



RESEARCH NOTE

Analysis of removal of cadmium by action of immobilized *Chlorella* sp. micro-algae in alginate beads [version 1; referees: 2 approved]

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v1 First published: 15 Jan 2018, 7:54 (doi: [10.12688/f1000research.13527.1](https://doi.org/10.12688/f1000research.13527.1))
 Latest published: 15 Jan 2018, 7:54 (doi: [10.12688/f1000research.13527.1](https://doi.org/10.12688/f1000research.13527.1))

Abstract

Cadmium (Cd) is a metal that can negatively interfere with the metabolic systems of living beings. The objective of this work was to evaluate the capacity for cadmium removal in aqueous solutions by immobilized *Chlorella* sp. in calcium alginate beads. Beads without *Chlorella* sp. were used as a control. All the treatments were established in triplicate for 80 min, at four concentrations of cadmium (0, 20, 100 and 200 ppm), taking samples of aqueous solution every 10 min, to be read using atomic absorption equipment. The study determined that the treatment of alginate beads with immobilized *Chlorella* sp. removed 59.67% of cadmium at an initial concentration of 20 ppm, this being the best removal result.

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Referee Status:  

	Invited Referees	
	1	2
version 1 published 15 Jan 2018	 report	 report

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Author roles: Valdez C: Data Curation, Methodology; Perengüez Y: Data Curation, Methodology; Mátyás B: Supervision, Writing – Review & Editing; Guevara MF: Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

How to cite this article: Valdez C, Perengüez Y, Mátyás B and Guevara MF. **Analysis of removal of cadmium by action of immobilized *Chlorella* sp. micro-algae in alginate beads [version 1; referees: 2 approved]** *F1000Research* 2018, 7:54 (doi: [10.12688/f1000research.13527.1](https://doi.org/10.12688/f1000research.13527.1))

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Grant information: The author(s) declared that no grants were involved in supporting this work.

First published: 15 Jan 2018, 7:54 (doi: [10.12688/f1000research.13527.1](https://doi.org/10.12688/f1000research.13527.1))

Introduction

Pollution from the use of metals as a consequence of waste generated by industries is a constant concern, since they may end up being transferred to the environment^{1,2}. The need to develop technologies for the remediation of water contaminated with cadmium should be considered as a priority in Ecuador. The objective of this study was to evaluate the capacity for cadmium removal in aqueous solutions by immobilized *Chlorella* sp. in calcium alginate beads. This study used the micro-algae *Chlorella* sp., for the bio-removal of cadmium, since the use of cells of *Chlorella* sp. immobilized in beads of alginate has been successfully exploited³ and its potential use has been established in the bioremediation of contaminants such as metals⁴, nutrients⁵ and other industrial pollutants⁶.

Methods

Alginate beads

The assay was carried out with aqueous solutions of cadmium. This solution was placed together with the alginate beads in Florence flasks. Every 10 minutes a sample of the aqueous solution was taken (10 mL approximately) with a glass pipette, and each sample was placed in a test tube. The concentration of metal was measured by aspiration atomic absorption spectrophotometer.

To make the control beads, the procedure described in a previous work was followed⁷. Briefly, 2 grams of sodium alginate (Loba Chemie, high density) were dissolved in 200 mL of ultra-pure water to obtain a homogeneous mixture, adjusting the pH between 7.8–8.0. pH was adjusted by addition of 0.1 molar sodium hydroxide or 0.1 molar hydrochloric acid, in sodium alginate paste (homogeneous mixture). One drop at a time was slowly poured into a 1% w/v calcium chloride solution with constant stirring.

For the immobilization of *Chlorella* sp. in alginate beads, liquid cultures were used. The volume of culture to be used was established to reach a concentration of 25×10^6 cells mL per each mL of alginate. For the preparation of the beads, 2 g of sodium alginate were dissolved in microalgae culture, as above.

Removal of cadmium with alginate beads

Experimental liquids were placed in 12 Florence Flasks of 500 mL capacity, inside which were placed 50 g of alginate beads without immobilized *Chlorella* sp. and 200 mL of cadmium solution at different concentrations (0, 20, 100 and 200 ppm). Each concentration was performed in triplicate, assay was evaluated during 80 minutes, aqueous solution samples were taken at 10 min intervals. Each Florence flask was placed on a shelf in the laboratory, the location of each bead on this surface was performed randomly. The flasks were subjected to constant aeration with air pressure of 0.012 MPa for 80 min. Every 10 minutes, an aqueous solution sample was taken to measure the concentration of metal present. These were the control treatments.

For the experimental samples, 50 g of alginate beads with immobilized *Chlorella* sp. were placed in each flask with the same concentrations of cadmium solution as the controls. These

experiments were also performed in triplicate. These experimental samples were used to establish whether the addition of *Chlorella* sp. in the beads, increases or decreases the alginate removal activity.

The determination of metal concentration in the solutions was measured with a VARIAN direct aspiration atomic absorption spectrophotometer (VARIAN. Model: SpectrAA 55, Wavelength: 326.1 nm), using air-acetylene flame with cadmium lamp, and results are presented as % of removal of cadmium.

Viability of micro-algae exposed to cadmium

For viability tests, color change of the cultures exposed to cadmium was evaluated. For this purpose 100 mL of liquid *Chlorella* cultures were used (25×10^6 cells/mL) and they were subjected to different concentrations of Cd (0, 20, 100 and 200 ppm), in triplicate. The color change was monitored at the first hour of treatment, at 24 hours and at 7 days.

Results and discussion

Viability of micro-algae exposed to cadmium

Color changes were observed in the solutions (Figure 1, Dataset 1 and Dataset 2) of free micro-algae cells exposed to different concentrations of cadmium, which is a product of the toxicity of the metal, while the solutions without the presence of metal maintained a green color, characteristic of *Chlorella* sp.⁷

It was determined that *Chlorella* sp. exposed to a concentration of 20 ppm cadmium at 60 minutes of contact does not react, and shows no noticeable changes in coloration. On the other hand, the micro-algae did not show resistance to concentrations 100 and 200 ppm of Cd, revealing that the cells did not remain viable due to the color variation and high toxicity of the metal used, going from green to a grayish brown in the first 60 minutes of the start of the trial. These results show to decrease algal growth and inhibit photosynthesis⁸ 100 ppm or higher.

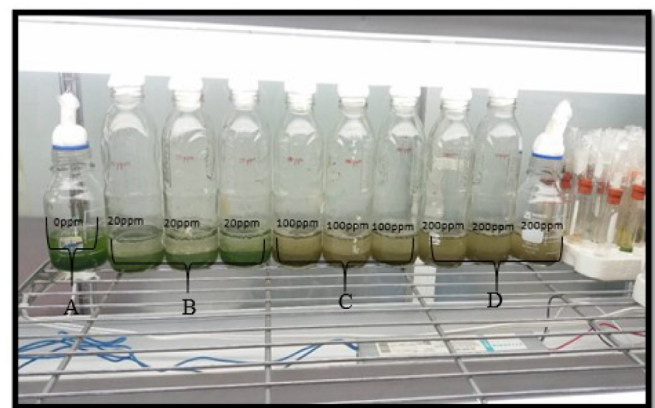


Figure 1. Viability tests of *Chlorella* sp. subjected to different concentrations of Cd, at 60 minutes, (A) 0 ppm Cd, (B) 20 ppm Cd, (C) 100 ppm Cd, (D) 200 ppm Cd. Green color means that algae remain viable, brown color means that *Chlorella* loses viability.

Dataset 1. Percentage of Cd in aqueous solution at 80 minutes<http://dx.doi.org/10.5256/f1000research.13527.d190266>**Dataset 2. Raw data for the percentage removal of cadmium with and without *Chlorella* alginate beads at all concentrations and triplicate experiments**<http://dx.doi.org/10.5256/f1000research.13527.d190281>**Removal of cadmium with alginate beads**

It is verified that the best treatment for Cd removal is the one corresponding to alginate beads with immobilized *Chlorella* sp. at the concentration of 20 ppm of Cd at 80 min. It was determined that at low metal concentrations the micro-algae enhances its removal capacity achieving a removal percentage of 59.67% with *Chlorella* sp., being significantly higher (p value <0.001) than the removal presented without *Chlorella* sp. (55.56%), explained by the viability process that showed that *Chlorella* sp. can withstand the toxicity of the metal for periods of at least 60 min at concentrations of 20 ppm (Figure 2 and Figure 3). As demonstrated by 9, micro-algae work better at lower concentrations of metal.

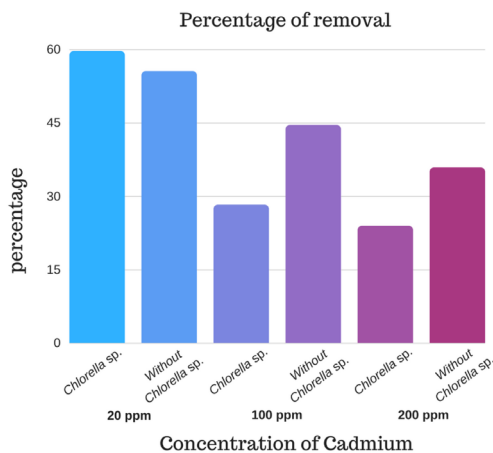


Figure 2. Comparison of the percentage of cadmium with different treatments carried out: alginate beads without *Chlorella* sp. (Without *Chlorella*), and alginate beads with *Chlorella* sp. (*Chlorella*).

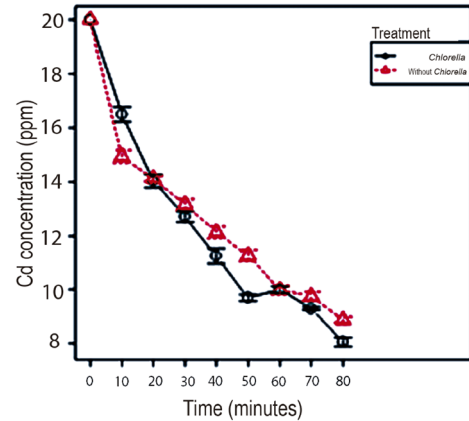


Figure 3. Variation of the concentration of Cd in the aqueous solution with respect to the time during the process of removal of 20 ppm of metal by the action of alginate beads with *Chlorella* sp. and without *Chlorella* sp.

Chlorella sp. immobilized in alginate beads could be used for bioremediation processes of cadmium at low concentrations of the metal, since the presence of viable biomass of the micro-algae potentiates the removal capacity of the alginate. Non-viable *Chlorella* sp., because of the high concentration of cadmium (100 and 200 ppm), decreases its removal capacity, yielding better results in beads treated without *Chlorella* sp. Thus, the alginate matrix without micro-algae could be used effectively in Cd removal processes at high metal concentrations.

Data availability

Dataset 1. Percentage of Cd in aqueous solution at 80 minutes. DOI, [10.5256/f1000research.13527.d190266](https://doi.org/10.5256/f1000research.13527.d190266)¹⁰

Dataset 2. Raw data for the percentage removal of cadmium with and without *Chlorella* alginate beads at all concentrations and triplicate experiments DOI, [10.5256/f1000research.13527.d190281](https://doi.org/10.5256/f1000research.13527.d190281)¹¹

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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[Data Source](#)
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[Data Source](#)

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Version 1

Referee Report 24 January 2018

doi:[10.5256/f1000research.14689.r29834](https://doi.org/10.5256/f1000research.14689.r29834)



Bernadett Gálya

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In my opinion, this article is well editable and easy to follow. The scope of each chapter is appropriate. In the Introduction, they briefly presented the problem behind the study. The study design is appropriate and the work is technically sound. They cited 11 references which is strongly connected to this topic. These are from 1998 to 2018, all in all, current literature was used. The method was described in detail, thus it is easy to replicate. They created three main parts in the methods. In my opinion is fine, but I suggest that the third subchapter should be the test used during the experiment. Otherwise, the materials, repetition and measurement which they are used are appropriate. The charts that are used are clear and easy to follow these in the text. In this article, the statistical analyses is applicable. May I suggest, that in the future they should use some other statistical test to compare different treatments (such as Tukey or Duncan-test). All source data are underlying the results available to ensure full reproducibility. The conclusions are drawn adequately supported by the results. Summary, this article is clear, easy to follow, used appropriate methods and scientifically based. Thus, it will be very useful to solve the examined problem.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

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Yes

If applicable, is the statistical analysis and its interpretation appropriate?

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Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Environmental technologies

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 23 January 2018

doi:10.5256/f1000research.14689.r29838



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The problems of pollution with heavy metals is a serious environmental issue. The use of microalgae supported on different substrates is an eco-biotechnological variant of great importance in sustainable conditions. The work is clear, concise and develops a simple and applicable strategy in field and laboratory conditions. The utilization of alginate beds (Ca²⁺) is a great technological solution

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

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Competing Interests: No competing interests were disclosed.

Referee Expertise: Applied chemical ecology and applied organic chemistry

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